

Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) **EP 1 099 760 A1**

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
16.05.2001 Bulletin 2001/20

(51) Int Cl.7: **C12N 15/32, C12N 15/62,
C07K 14/325, A01H 5/00,
A01N 63/00**

(21) Application number: **99203723.4**

(22) Date of filing: **09.11.1999**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE**
Designated Extension States:
AL LT LV MK RO SI

(72) Inventors:
• **de Maagd, Rudolf Aart**
6708 HL Wageningen (NL)
• **Bosch, Hendrik Jan**
3572 ZM Utrecht (NL)

(71) Applicant: **CENTRUM VOOR
PLANTENVEREDELINGS- EN
REPRODUKTIEONDERZOEK (CPRO)**
6708 PB Wageningen (NL)

(74) Representative: **De Hoop, Eric**
Octrooibureau Vriesendorp & Gaade
P.O. Box 266
2501 AW Den Haag (NL)

(54) **Bacillus thuringiensis Cry1Ia-Cry1Ba hybrid toxins**

(57) *Bacillus thuringiensis* hybrid toxin fragment comprising structural domains I, II and III in this order starting from the N-terminal, wherein the domains are derived from at least two different Cry proteins, domain I is domain I of any *Bacillus thuringiensis* Cry protein or

a part of said domain or a peptide substantially similar to said domain, domain II is domain II of Cry1Ia or a part of said domain or a peptide substantially similar to said domain, and domain III is domain III of Cry1Ba or a part of said domain or a peptide substantially similar domain.

EP 1 099 760 A1

Descripti n

1.0. Field of the invention

[0001] The present invention relates to hybrid toxin fragments, and toxins comprising them, derived from *Bacillus thuringiensis* insecticidal crystal proteins.

1.1. Description of the related art

[0002] *Bacillus thuringiensis* (hereinafter B.t.) is a gram-positive bacterium that produces insecticidal crystal proteins during sporulation. The crystal (Cry) proteins form a large (over 160 and growing) family of homologous proteins with unique specificities. Each protein is active against only one or a few insect species. Most reported proteins are active against lepidopterans, with a smaller number showing activity against diptera or coleoptera as reviewed in Schnepf *et al.*, 1998). While initially the Cry proteins were classified according to their activity against one of the abovementioned insect orders (Höfte and Whiteley, 1989), the more recent, now commonly accepted classification is based on amino acid homology (Crickmore *et al.*, 1998).

[0003] The mode of actions of crystal proteins has been partially elucidated. After ingestion by the insect larvae, crystals dissolve in the midgut environment, releasing the proteins as protoxins of 70-140 kDa. The solubilized protoxins are subsequently processed ("activated") by midgut proteases, resulting in a protease-resistant fragment of about 65 kDa, which is the active toxin. The toxin binds to receptors on epithelial cells of the insect midgut and penetrates the membrane. This eventually leads to lysis of the cells and death of the larvae.

[0004] The activity range of a particular delta-endotoxin is to a large extent determined by the occurrence of receptors on the midgut cells of the insect, although solubilization efficiency and proteolytic activation are also factors involved. The importance of binding to receptors is further exemplified by the decrease in binding occurring in many instances of resistance to Cry proteins (Ferre *et al.*, 1995).

[0005] Structure determination by X-ray cristallography has shown that three different Cry proteins, and probably all Cry proteins, share a common three domain-structure (Li *et al.*, 1991; Grochulski *et al.*, 1995; Morse *et al.*, 1998). If projected on Cry1 sequences, domain I runs from about amino acid residue 28 to 260, domain II from about 260 to 460 and domain III from about 460 to 620. Since the various toxins have different lengths, the borders of the domains can be defined only approximately. A person skilled in this art will be able to find the borders for the various toxins by comparing the amino acid sequences. The N-terminal domain I consists of 7 α -helices and is considered to be inserting (partially) into the target membrane, and forming part of the pore that eventually kills the insect gut epithelial cells. Both domain II and the C-terminal domain III are very variable, and have been shown to determine activity against specific insects. Although it is not yet clear how these domains individually or acting together may determine specificity, there is strong evidence that both can be involved in binding to (putative) receptors (Lee *et al.*, 1995; Dean *et al.*, 1996; de Maagd *et al.*, 1996b; de Maagd *et al.*, 1999).

[0006] Exchange of domain III between toxins by *in vivo* recombination of their encoding genes may not only alter specificity of a toxin, but can also result in a hybrid toxin with superior toxicity for certain insects (Bosch *et al.*, 1994; de Maagd *et al.*, 1996a). Intl. Pat. Appl. Publ. No. WO 95/06730 discloses the construction of a hybrid delta-endotoxin consisting of domains I and II of Cry1E, and domain III and protoxin-specific fragment of Cry1C. In bioassays, this hybrid as purified after production in *E. coli* is active against *Manduca sexta*, *Spodoptera exigua*, and *Mamestra brassicae*. When expressed in and purified from a recombinant *Bacillus thuringiensis* strain, the 1E/1C-hybrid was 1.5 times as active as the most active natural toxin against *S. exigua*, Cry1C. The abovementioned patent application also describes a hybrid delta-endotoxin consisting of domains I and II of Cry1Ab, and domain III of Cry1C. When purified from a recombinant *Bacillus thuringiensis* strain, this Cry1 Ab/Cry1 C-hybrid was approximately 6.6 times as toxic as Cry1C (de Maagd *et al.*, 1996a). Intl. Pat. Appl. Publ. No. WO 98/22595 discloses a number of hybrid toxins consisting of different combinations of fragments of Cry1Ab, Cry1Ac, Cry1C or Cry1F, of which some have improved acitivity against important pest larvae and/or an extended activity spectrum.

[0007] Producing succesfull hybrids is not easy. From the prior art it appears that only a very limited number of hybrids have an improved activity or a broader host-range specificity. In addition there are many possibilities for recombinantly-engineered crystal proteins, in view of the big number of different crystal proteins (more than 160). Further, the effect of the hybrids produced is not predictable.

2.0 Summary of the invention

[0008] Proteins of the Cry3 (Herrnstadt *et al.*, 1986; McPherson *et al.*, 1988), Cry7 (Lambert *et al.*, 1992) and Cry8 (Sato *et al.*, 1994) classes were found to be active against insects of the order Coleoptera (beetles). Cry3A is the singlemost active protein for the important potato pest Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say).

[0009] It is known that resistance to the endotoxins may occur. Accordingly, resistance to Cry3A may occur in CPB in response to its exposure to Cry3A in transgenic potato. Actually, resistant CPB has already been disclosed. Therefore, it is desirable to have an alternative or replacement for Cry3A.

[0010] The present inventors have found specific hybrid crystal proteins which have a high activity against CPB and therefore can be used as a replacement for Cry3A. The hybrids of the invention comprise domains derived from Cry1Ia and Cry1Ba.

[0011] Cry1 proteins are generally active against lepidopterans (larvae of moths and butterflies). Cry1B and Cry1I have been shown to also have some activity against coleopterans, among which CPB, although their toxicity for CPB is much lower than that of Cry3A (Tailor *et al.*, 1992; Bradley *et al.*, 1995).

[0012] The toxicity of the hybrid crystal proteins of the invention against CPB, as compared to the parental proteins Cry1Ba and Cry1Ia, is surprisingly higher. Additionally a number of the hybrids of the invention have retained the high activity against some Lepidopterans, making them toxins with meaningful dual activity for Coleopterans and Lepidopterans.

[0013] According to the present invention there is provided a *B. t.* hybrid toxin fragment comprising structural domains I, II and III in this order starting from the N-terminal, wherein the domains are derived from at least two different Cry proteins, domain I is domain I of any of *B. t.* Cry protein or a part of said domain or a peptide substantially similar to said domain, domain II is domain II of Cry1Ia or a part of said domain or a peptide substantially similar to said domain, and domain III is domain III of Cry1Ba or a part of said domain or a peptide substantially similar to said domain. Preferred is a fragment which comprises domain I of Cry1Ia or Cry1Ba or a part of said domain or a peptide substantially similar to said domain, domain II of Cry1Ia or a part of said domain or a peptide substantially similar to said domain, and domain III of Cry1Ba or a part of said domain or a peptide substantially similar to said domain.

[0014] The term "or a peptide substantially similar to said domain" should be understood to mean a peptide having an amino acid sequence which is at least 85% similar to the sequence of the domain. It is preferred that the degree of similarity is at least 90%.

[0015] In the context of the present invention, two amino acid sequences with at least 85% or 90% similarity to each other have at least 85% or 90% identical or conservatively replaced amino acid residues in a like position when aligned optimally allowing for up to 6 gaps with the proviso that in respect of the gaps a total not more than 15 amino acid residues are affected. For the purpose of the present invention conservative replacements may be made between amino acids with the following groups:

1. Serine and Threonine;
2. Glutamic acid and Aspartic acid;
3. Arginine and Lysine
4. Asparagine and Glutamine
5. Isoleucine, Leucine, Valine, and Methionine;
6. Phenylalanine, Tyrosine, and Tryptophan
7. Alanine and Glycine

By "or a part of said domain" is meant a peptide comprised by the said domain and having at least 80% of the consecutive sequence thereof.

[0016] It is most particularly preferred that the toxin fragment according to the invention comprises either:

1. An amino acid sequence from about amino acid 20 to about amino acid 641 in SEQ ID NO:2 or
2. An amino acid sequence from about amino acid 20 to about amino acid 632 in SEQ ID NO:4.

[0017] The invention also includes a hybrid toxin comprising the above disclosed fragment or a toxin at least 85% similar to such a hybrid toxin which has substantially similar insecticidal activity.

[0018] The hybrid toxin comprises the three structural domains as defined above and generally may comprise a pro-toxin segment at the carboxyl end, which is not toxic and is thought to be important for crystal formation. SEQ ID NO: 2 and 4 comprise such pro-toxin segment. Further, the hybrid toxin may comprise a lead sequence, being amino acids 1-19 in SEQ ID NO:2 and 4.

[0019] The invention still further includes pure proteins which are at least 90% identical to the toxin fragments or hybrid toxins according to the invention.

[0020] The invention still further includes recombinant DNA comprising a sequence encoding a protein having an amino acid sequence of the above disclosed toxins or fragments thereof.

[0021] In a preferred embodiment the invention provides recombinant DNA comprising the sequence as shown in SEQ ID NO:1 or 3 or DNA similar thereto encoding a substantially similar protein.

[0022] In a more preferred embodiment the invention provides recombinant DNA comprising the sequence from

about nucleotide 170 to about 1929 in SEQ ID NO:1 or from about nucleotide 147 to about 1896 in SEQ ID NO:3.

[0023] By similar DNA is meant a test sequence which is capable of hybridizing to the inventive recombinant sequence. When the test and inventive sequences are double stranded the nucleic acid constituting the test sequence preferably has a T_m within 20°C of that of the inventive sequence. In the case that the test and inventive sequences are mixed together and denatured simultaneously, The T_m values of the sequences are preferably within 10°C of each other. More preferably the hybridization is performed under stringent conditions, with either the test or inventive DNA preferably being supported. Thus either a denatured test or inventive sequence is preferably first bound to a support and hybridization is effected for a specified period of time at a temperature of between 50 and 70 °C in double strength citrate buffered saline containing 0.1 % SDS followed by rinsing of the support at the same temperature but with a buffer having a reduced SSC concentration. Depending upon the degree of stringency required, and thus the degree of similarity of the sequences, such reduced concentration buffers are typically single strength SSC containing 0.1 % SDS. Sequences having the greatest degree of similarity are those the hybridization of which is least affected by washing in buffers of reduced concentration. It is most preferred that the test and inventive sequences are so similar that the hybridization between them is substantially unaffected by washing or incubation in one tenth strength sodium citrate buffer containing 0.1% SDS.

[0024] The recombinant DNA may further encode a protein having herbicide resistance, plant growth-promoting, anti-fungal, anti-bacterial, anti-viral and/or anti-nematode properties. In the case that the DNA is to be introduced into a heterologous organism it may be modified to remove known mRNA instability motifs (such as AT rich regions) and polyadenylation signals, and/or codons which are preferred by the organism into which the recombinant DNA is to be inserted may be used so that expression of the thus modified DNA in the said organism yields substantially similar protein to that obtained by expression of the unmodified recombinant DNA in the organism in which the protein components of the hybrid toxin or toxin fragments are endogenous.

[0025] The invention still further includes a DNA sequence which is complementary to one which hybridizes under stringent conditions with the recombinant DNA according to the invention.

[0026] Also included in the present invention are: a vector containing such a recombinant (or complementary thereto) DNA sequence; a plant or micro-organism which includes, and enables expression of such DNA; plants transformed with such DNA; the progeny of such plants which contain the DNA stably incorporated and heritable in a Mendelian manner, an/or the seeds of such plants and such progeny. The invention still further includes protein derived from expression of the said DNA, and insecticidal protein produced by expression of the recombinant DNA within plants transformed therewith.

[0027] The invention still further includes an insecticidal composition containing one or more of the toxin fragments or toxins comprising them according to the invention; a process for combatting insects which comprises exposing them to such fragments or toxins or compositions, and an extraction process for obtaining insecticidal proteins from organic material containing them comprising submitting the material to maceration and solvent extraction.

[0028] The invention will be further apparent from the following description, which describes the production of Bt hybrid toxin fragments according to the invention, taken in conjunction with the associated drawings and sequence listings.

[0029] A person skilled in the art may use different methods to construct the hybrid toxin genes of the invention. Domain encoding regions may be exchanged between two homologous, though different genes by exchanging fragments through restriction enzyme digestion and subsequent ligation, if the same restriction enzyme recognition sites are present at the same position in the two genes. If suitable restriction enzyme recognition sites are not available, new sites can be created at homologous positions in the two genes by various methods of site-directed mutagenesis, as described in the examples below for construction of a common *RsrII*-site using DNA oligomers SEQ ID: 9 and SEQ ID: 10.

[0030] Alternatively, fragments that are to be combined in a hybrid toxin gene may be produced by PCR-mediated amplification using the original genes as templates, taking care that the ends of the fragments contain compatible restriction enzyme digestion sites. Furthermore, a hybrid toxin encoding DNA fragment may be produced by *in vitro* recombination between overlapping, partially homologous DNA fragments as performed in so-called DNA shuffling experiment (Cramer et al, 1998; Zhao et al., 1998). Also, hybrid toxin encoding DNA fragments may be produced by *in vivo* recombination between two homologous DNA fragments that have been cloned in tandem on a single plasmid (Bosch et al., 1994), followed by screening for the desired recombination events.

3.0 Brief description of the drawings

[0031] Figure 1 shows the sequence of the relevant part of the *cry1Ba* (top) and *cry1Ia* (bottom) genes, respectively, aligned with the respective oligomers (SEQ ID NO:9 and 10) used for mutagenesis in order to produce a common *RsrII* restriction enzyme recognition site in both genes. Mutated nucleotides are indicated by asterisks and the newly produced *RsrII* recognition sites are underlined.

[0032] Figure 2 shows both the *cry1Ia* gene (solid bar) as well as the *cry1Ba* fragment (open bar) present as they occur in expression vectors pSN18 and pSN17, respectively. Both genes are inserted in the pKK233-2 derived expression vector for *E. coli*, pBD12. For construction of pSN18 the 3' part of the *cry1Ba* gene, encoding the C-terminal part of the protoxin-specific fragment, was replaced by the corresponding part of the *cry1Ca* gene (base pairs 2038-3627; dashed bar). The positions of the common *NcoI* and *MunI* restriction sites, as well as of the *RsrII* sites derived by mutagenesis, and the *BstXI*-site used for exchange of the 3' end of the *cry1Ba* gene are indicated, with nucleotide position numbers below the respective genes.

[0033] Figure 3 shows in a scheme the construction of hybrid gene SN15 from *cry1Ba* (pSN17) and *cry1Ia* (pSN18), and the subsequent construction of hybrid gene SN19 from *cry1Ba* (pSN17) and pSN15.

[0034] Figure 4 shows the alignment of *cry1Ba* and *cry1Ia* nucleotide sequences, as present in plasmids pSN17 and pSN18, respectively, around the junctions between the domain II and III (A) and between domain I and II (B). Nucleotide sequences as present in the hybrid genes of SN15 and pSN19 (A) or of pSN19 alone (B) are given in capitals, while identical nucleotides in the DNA that is not present in SN15 and SN19 is represented by dots. The position of the common *RsrII* and *MunI* recognition sites are underlined. The site of crossover in the hybrids is represented by the shift of the fully written out nucleotide sequence from top strand to bottom strand in this recognition sites. The amino acid sequence of the resulting hybrid proteins is given in three-letter code below each alignment.

4.0 Brief description of the sequence identifiers

[0035] SEQ ID NO:1 shows the nucleotide sequence of the hybrid protoxin gene SN15.

[0036] SEQ ID NO:2 shows the amino acid sequence of the protein encoded by the gene SN15 shown in SEQ ID NO:1.

[0037] SEQ ID NO:3 shows the nucleotide sequence of the hybrid protoxin gene SN19.

[0038] SEQ ID NO:4 shows the amino acid sequence of the protein encoded by the gene SN19 shown in SEQ ID NO:3.

[0039] SEQ ID NO:5 shows the nucleotide sequence of the modified *cry1Ba* gene as used in expression vector pSN17.

[0040] SEQ ID NO:6 shows the amino acid sequence of the protein encoded by the *cry1Ba* gene shown in SEQ ID NO:5.

[0041] SEQ ID NO:7 shows the nucleotide sequence of the modified *cry1Ia* gene as used in expression vector pSN18.

[0042] SEQ ID NO:8 shows the amino acid sequence of the protein encoded by the *cry1Ia* gene shown in SEQ ID NO:7.

[0043] SEQ ID NO:9 shows the nucleotide sequence of the oligomer used for mutagenesis of the *cry1Ba* gene.

[0044] SEQ ID NO:10 shows the nucleotide sequence of the oligomer used for mutagenesis of the *cry1Ia* gene.

5.0 Examples

5.1 DNA manipulations

[0045] All recombinant DNA techniques are as described by Ausubel et al. (1997). Mutagenesis, restriction enzyme digestion and ligation are performed according the instructions of the manufacturers. DNA sequencing is performed by the dideoxytriphosphate method with fluorescent dyes attached to the dideoxynucleotides. Analysis is automated by using an Applied Biosystems 370A nucleotide sequence analyzer.

5.2 Expression vectors

[0046] All Cry protein expression vectors are based on pBD12, a derivative of pKK233-2 (Bosch et al., 1994). For expression of Cry3Aa protein, the full *cry3Aa* gene is cloned into pBD12, giving expression plasmid pMH10. For cloning purposes both *cry1Ba* as well as *cry1Ia* are mutagenized in order to contain a *NcoI*-site overlapping with the start codon. For production of Cry1Ba protein, a *NcoI*-*BstXI* (bases 1-1977) fragment of *cry1Ca* in pBD150 (Bosch et al., 1994) is replaced by the corresponding fragment of *cry1Ba* (bases 1-2037), resulting in *cry1Ba* expression vector pMH19. pMH19 therefore contains the 5' active toxin encoding part of Cry1Ba and 3' protoxin specific part of Cry1Ca. Cry1Ia expression vector pBD172 contains the full *cry1Ia* gene with the *SpeI*-site (base 2180) fused to the *SpeI*-site in the polylinker of pBluescript SK⁺.

5.3 Mutagenesis of *cry1Ba* and *cry1Ia*

[0047] In order to be able to directly exchange the domain III encoding regions between *cry1Ba* and *cry1Ia*, a new

common restriction enzyme recognition site is made in both genes by site directed mutagenesis. Complementary mutagenic oligonucleotide pairs are used to create unique *RsrII*-sites at positions 1464 and 1488 of *cry1Ba* (pMH19) and *cry1Ia* (pBD172), respectively (see Fig. 1), using the QuickChange™ kit (Stratagene). These mutations do not change the encoded proteins. This results in two new expression plasmids, pSN17 (*Cry1Ba*, Seq. ID NO: 5) and pSN18 (*Cry1Ia*, Seq. ID NO: 7), respectively (Fig. 2). The unique *RsrII* restriction sites at the border of the domain II and domain III encoding regions, together with the common *MunI*-sites at the border between the domain I and domain II encoding regions allowed simple swapping of domain encoding regions between the two genes.

5.4 Construction of hybrid toxins

[0048] Fig. 3 schematically shows the construction of two novel hybrid toxins. Both pSN18 and pSN17 are digested with *NcoI* and *RsrII*. Subsequently, the 1488 base fragment of pSN18, containing the domain I and II encoding fragment of *cry1Ia* is ligated into the corresponding sites of the pSN17-derived fragment containing the 3'portion of *cry1Ba*. This results in plasmid pSN15 encoding a 1Ia/1Ia/1Ba-hybrid (Seq. ID NO:1). The nucleotide sequence of the cross-over region with the encoded amino acid sequence is shown in Fig. 4A. Subsequently a *NcoI-MunI* (base 1-896) fragment encoding domain I of *Cry1Ia* from pSN15 is replaced by the corresponding fragment encoding domain I of *Cry1Ba*, derived from pSN17. This results in 1Ba/1Ia/1 Ba-hybrid encoding plasmid pSN19 (Seq. ID. NO:3). The nucleotide sequence of the crossover region with the encoded amino acid sequence is shown in Fig. 4B.

5.5 Protein isolation and insect bioassays.

[0049] For large-scale production, all parental and hybrid protoxins are expressed in *E. coli* strain XL-1 and extracted as described earlier (Bosch *et al.*, 1994). Solubilized protoxins are dialyzed overnight in 25 mM NaHCO₃, 100 mM NaCl, pH10. Protein concentrations are estimated by SDS-PAGE (sodium dodecylsulphate polyacrylamide gel electrophoresis). To test toxicity to Colorado potato beetle (CPB), leaflets of greenhouse grown potato cultivar Desiree are dipped in toxin dilutions in water containing 0.01 % Tween-20. After drying of the leaves to the air they are transferred to petri dishes and 10 neonate CPB larvae are placed on each leaf. After incubation for two days at 28°C, the leaves are replaced by fresh leaves dipped in identical protoxin dilutions. Mortality is scored after 4 days. LC₅₀ (concentration with 50% mortality) and 95% fiducial limits are determined by Probit analysis of results from three or more independent experiments, using the PoloPC computer program (Russel *et al.*, 1977).

5.6 Toxicity of wild type and hybrid proteins to Colorado potato beetle

[0050] Table 1 shows the toxicity of the hybrid proteins SN15 and SN19 against Colorado potato beetle (CPB), as compared to the parental proteins *Cry1Ba* and *Cry1Ia*, and to the most CPB-active natural toxin available, *Cry3A*. 1Ia/1Ia/1Ba-hybrid SN15 shows slightly higher toxicity (lower LC₅₀) than its best parental toxin, *Cry1Ia*, on a per weight basis. When considering that the molecular weight of the SN15 protein is considerably larger than that of *Cry1Ia*, it follows that SN15 performs even better on a per mol basis. 1Ba/1Ia/1Ba-hybrid protein SN19 is even considerably more toxic than SN15.

Table 1.

Toxicity of wild type and hybrid protoxins to Colorado potato beetles.				
Protoxin	LC ₅₀ ^a	95% fiducial limits ^a	MW ^b	Relative toxicity ^c
MH10 (<i>Cry3A</i>)	1.8	1.4-2.5	74.0	100
SN17(<i>Cry1Ba</i>)	142	105-198	137.4	1
SN18(<i>Cry1Ia</i>)	34	23-47	81.3	6
SN15	22	14-35	138.0	15
SN19	8	5-11	137.2	42

^aConcentration in microgram per milliliter of dipping solution. LC₅₀: concentration which leads to 50% mortality;

^bApproximate molecular weight in kiloDaltons.

^cRelative toxicity on molar basis in percents, with toxicity of *Cry3A* set at 100%.

Although the present invention has been particularly described for the production of SN15 and SN19 hybrid toxins, the

skilled scientist will appreciate that other hybrid toxins with improved insecticidal characteristics may be produced according to the invention. Hybrid toxins containing the combination of Cry1Ia domain II and Cry1Ba domain III, with various combinations of domain I and C-terminal extensions may be made. Moreover, the gene encoding SN15, SN19 and/or other hybrids may be transferred into strains of B.t. and/or integrated into the chromosome of strains of B.t., of other bacteria or be introduced into plant genomes to provide for in situ insecticidal activity within the plant per se. In this regard, it is particularly preferred that the recombinant DNA encoding the toxins is modified in that sequences which are detrimental to high level expression in plants are removed and in that codons which are preferred by the plant are used. This should lead to production in the plant of a substantially similar protein to that obtained by expression of the unmodified recombinant DNA in the organism in which the protein components of the hybrid toxins or toxin fragments are endogenous.

6.0 References

[0051] The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

- Intl. Pat. Appl. Publ. No. WO 95/06730, published 9 March 1995
 Intl. Pat. Appl. Publ. No. WO 98/22595, published 28 May 1998
 Ausubel, F. *et al.* (1997) *Current Protocols in Molecular Biology*. John Wiley & Sons.
- Bosch, D., Schipper, B., van der Kleij, H., de Maagd, R. and Stiekema, W. (1994) Recombinant *Bacillus thuringiensis* crystal proteins with new properties: possibilities for resistance management. *Biototechnology*, **12**, 915-918.
- Bradley, D., Harkey, M.A., Kim, M.K., Biever, K.D. and Bauer, L.S. (1995) The insecticidal CryIB crystal protein of *Bacillus thuringiensis* ssp. *thuringiensis* has dual specificity to Coleopteran and Lepidopteran larvae. *Journal of Invertebrate Pathology*, **65**, 162-173.
- Cramer, A., S. A. Raillard, E. Bernudez, and W. P. C. Stemmer. 1998. DNA shuffling of a family of genes from diverse species accelerates directed evolution. *Nature*. 391(6664):288-291.
- Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., VanRie, J., Lereclus, D., Baum, J. and Dean, D.H. (1998) Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and molecular biology reviews*, **62**, 807&.
- de Maagd, R.A., Bakker, P.L., Masson, L., Adang, M.J., Sangadala, S., Stiekema, W. and Bosch, D. (1999) Domain III of the *Bacillus thuringiensis* delta-endotoxin Cry1Ac is involved in binding to *Manduca sexta* brush border membranes and to its purified aminopeptidase N. *Molecular microbiology*, **31**, 463-471.
- de Maagd, R.A., Kwa, M.S.G., van der Kleij, H., Yamamoto, T., Schipper, B., Vlak, J.M., Stiekema, W.J. and Bosch, D. (1996a) Domain III substitution in *Bacillus thuringiensis* delta-endotoxin CryIA(b) results in superior toxicity for *Spodoptera exigua* and altered membrane protein recognition. *Applied & Environmental Microbiology*, **62**, 1537-1543.
- de Maagd, R.A., van der Kleij, H., Bakker, P.L., Stiekema, W.J. and Bosch, D. (1996b) Different domains of *Bacillus thuringiensis* delta-endotoxins can bind to insect midgut membrane proteins on ligand blots. *Applied & Environmental Microbiology*, **62**, 2753-2757.
- Dean, D.H., Rajamohan, F., Lee, M.K., Wu, S.J., Chen, X.J., Alcantara, E. and Hussain, S.R. (1996) Probing the mechanism of action of *Bacillus thuringiensis* insecticidal proteins by site directed mutagenesis: a minireview. *Gene*, **179**, 111-117.
- Ferre, J., Escriche, B., Bel, Y. and van Rie, J. (1995) Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins. *FEMS Microbiology Lett.*, **132**, 1-7.
- Grochulski, P., Masson, L., Borisova, S., Pusztai-Carey, M., Schwartz, J.L., Brousseau, R. and Cygler, M. (1995) *Bacillus thuringiensis* CryIA(a) insecticidal toxin - crystal structure and channel formation. *Journal of Molecular Biology*, **254**, 447-464.
- Herrnstadt, C., Soares, G.G., Wilcox, E.R. and Edwards, D.L. (1986) A new strain of *Bacillus thuringiensis* with activity against coleopteran insects. *Biotechnology*, **4**, 305-308.
- Höfte, H. and Whiteley, H.R. (1989) Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.*, **53**, 242-255.
- Lambert, B., Hofte, H., Annys, K., Jansens, S., Soetaert, P. and Peferoen, M. (1992) Novel *Bacillus thuringiensis* insecticidal crystal protein with a silent activity against coleopteran larvae. *Applied And Environmental Microbiology*, **58**, 2536-2542.
- Lee, M.K., Young, B.A. and Dean, D.H. (1995) Domain III exchanges of *Bacillus thuringiensis* Cry1A toxins affect binding to different Gypsy Moth midgut receptors. *Biochemical and Biophysical Research Communications*, **216**, 306-312.
- Li, J., Carroll, J. and Ellar, D.J. (1991) Crystal structure of insecticidal delta-endotoxin from *Bacillus thuringiensis*

at 2.5 Å resolution. *Nature (London)*, **353**, 815-821.

McPherson, S., Perlak, F., Fuchs, R., Marrone, P., Lavrik, P. and Fischhoff, D. (1988) Characterization of the coleopteran-specific protein gene of *Bacillus thuringiensis* var. *tenebrionis*. *Biotechnology*, **6**, 61-66.

Morse, R.J., Powell, G., Ramalingam, V., Yamamoto, T. and Stroud, R.M. (1998) Crystal structure of Cry2Aa from *Bacillus thuringiensis* at 2.2 Å resolution: structural basis of dual specificity. In Iizuka, T. (ed.) *Vllth International Colloquium on Invertebrate Pathology and Microbial Control and IVth International Conference on Bacillus thuringiensis*. Sapporo, pp. 1-2.

Russel, R.M., Robertson, J.L. and Savin, N.E. (1977) POLO: a new computer program for Probit analysis. *ESA Bulletin*, **23**, 209-213.

Sato, R., Takeuchi, K., Ogiwara, K., Minami, M., Kaji, Y., Suzuki, N., Hori, H. and Asano, S. (1994) Cloning, heterologous expression, and localization of a novel crystal protein gene from bacillus-thuringiensis serovar japonensis strain buibui toxic to scarabaeid insects. *Curr Microbiol*, **28**, 15-19.

Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H. (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiology and molecular biology reviews*, **62**, 775-806.

Taylor, R., Tippet, J., Gibb, G., Pells, S., Pike, D., Jordan, L. and Ely, S. (1992) Identification and characterization of a novel *Bacillus thuringiensis* delta-endotoxin entomocidal to coleopteran and lepidopteran larvae. *Mol. Microbiol.*, **6**, 1211-1217.

Zhao, H. M., L. Giver, Z. X. Shao, J. A. Affholter, and F. H. Arnold. 1998. Molecular evolution by staggered extension process (StEP) in vitro recombination. *Nature biotechnology*. **16**(3):258-261.

EP 1 099 760 A1

SEQUENCE LISTING

5 <110> CPRO-DLO

<120> Bacillus thuringiensis hybrid toxins

10 <130> E158870

<140>

<141>

15 <160> 10

<170> PatentIn Ver. 2.1

20 <210> 1

<211> 3651

<212> DNA

<213> Artificial Sequence

25 <220>

<223> Description of Artificial Sequence: hybrid
protoxin gene SN15

30 <400> 1

atgaaactaa agaatcaaga taagcatcaa agtttttcta gcaatgcgaa agtagataaa 60

atctctacgg attcactaaa aaatgaaaca gatatagaat tacaaaacat taatcatgaa 120

gattgtttga aaatgtctga gtatgaaaat gtagagccgt ttgttagtgc atcaacaatt 180

35 caaacaggta ttggtattgc gggtaaaata cttggtaccc taggcgttcc ttttgaggga 240

caagtagcta gtctttatag ttttatctta ggtgagctat ggcctaaggg gaaaaatcaa 300

tgggaaatct ttatggaaca tgtagaagag attattaatc aaaaaatc aacttatgca 360

agaaataaag cacttacaga cttgaaagga ttaggagatg ccttagctgt ctaccatgat 420

tcgcttgaaa gttgggttgg aaatcgtaat aacacaaggg ctaggagtgt tgtcaagagc 480

40 caatatatcg cattagaatt gatgttcgtt cagaaactac cttcttttgc agtgtctgga 540

gaggaggtac cattattacc gatatatgcc caagctgcaa atttacattt gttgctatta 600

agagatgcat ctatttttgg aaaagagtgg ggattatcat cttcagaaat ttcaacattt 660

tataaccgtc aagtcgaacg agcaggagat tattcctacc attgtgtgaa atggtatagc 720

45 acagggtctaa ataacttgag gggtaaaaat gccgaaagtt gggtagcata taatcaattc 780

cgtagagaca tgactttaat ggtactagat ttagtggcac tatttccaag ctatgataca 840

caaattgtatc caattaaaac tacagcccaa cttacaagag aagtatatac agacgcaatt 900

gggacagtac atccgcatcc aagttttaca agtacgactt ggtataataa taatgcacct 960

tcgttctctg ccatagaggc tgctgttgtt cgaaacccgc atctactcga ttttctagaa 1020

50 caagttacaa ttacagctt attaagtcga tggagtaaca ctcagtatat gaatatgttg 1080

ggaggacata aactagaatt ccgaacaata ggaggaaact taaatatctc aacacaagga 1140

tctactaata cttctattaa tcctgtaaca ttaccgttca cttctcgaga cgtctatagg 1200

actgaatcat tggcagggct gaatctatct ttaactcaac ctgttaattg agtacctagg 1260

55 gttgatttcc attggaaatt cgtcacacac ccgacgcat ctgataattt ctattatcca 1320

gggtatgctg gaattgggac gcaattacag gattcagaaa atgaattacc acctgaagca 1380

EP 1 099 760 A1

acaggacagc caaattatga atcttatagt catagattat ctcatatagg actcatttca 1440
 gcatcacatg tgaagcatc ggtatattct tggacgcac gtagtgccga ccgtacgaat 1500
 acgattggac caaatagaat caccctaatc ccaatggtaa aagcatccga acttcctcaa 1560
 5 ggtaccactg ttgttagagg accaggattt actgggtggg atattcttcg aagaacgaat 1620
 actggtggat ttggaccgat aagagtaact gttaacggac cattaacaca aagatatcgt 1680
 ataggattcc gctatgcttc aactgtagat tttgatttct ttgtatcacg tggaggtact 1740
 actgtaaata attttagatt cctacgtaca atgaacagtg gagacgaact aaaatacggg 1800
 10 aattttgtga gacgtgcttt tactacacct tttactttta cacaaattca agatataatt 1860
 cgaacgtcta ttcaaggcct tagtggaagt ggggaagtgt atatagataa aattgaaatt 1920
 attccagtta ctgcaacctt cgaagcagaa tatgatttag aaagagcgca agaggcgggtg 1980
 aatgctctgt ttactaatac gaatccaaga agattgaaaa cagatgtgac agattatcat 2040
 attgatcaag tatccaattt agtggattgt ttatcagatg aattttgtct ggatgaaaag 2100
 15 cgagaattgt ccgagaaagt caaacatgcg aagcgactca gtgatgagcg gaatttactt 2160
 caagatccaa acttcagagg gatcaataga caaccagacc gtggctggag aggaagtaca 2220
 gatattacca tccaaggagg agatgacgta ttcaaagaga attacgtcac actaccgggt 2280
 accgttgatg agtgctatcc aacgtattta tatcagaaaa tagatgagtc gaaattaaaa 2340
 20 gcttataccg gttatgaatt aagagggtat atcgaagata gtcaagactt agaaatctat 2400
 ttgatccggt acaatgcaaa acacgaaata gtaaattgtc caggcacggg ttccttatgg 2460
 ccgctttcag cccaaagtcc aatcggaag tgtggagaac cgaatcgatg cgcgccacac 2520
 cttgaatgga atcctgatct agattgttcc tgcagagacg gggaaaaatg tgcacatcat 2580
 25 tcccatcatt tcacctgga tattgatgtt ggatgtacag acttaaatga ggacttaggt 2640
 gtatgggtga tattcaagat taagacgcaa gatggccatg caagactagg gaatctagag 2700
 tttctcgaag agaaaccatt attaggggaa gcactagctc gtgtgaaaag agcggagaag 2760
 aagtggagag acaaacgaga gaaactgcag ttggaaacaa atattgttta taaagaggca 2820
 aaagaatctg tagatgcttt atttgtaaac tctcaatatg atagattaca agtggatacg 2880
 30 aacatcgcca tgattcatgc ggcagataaa cgcgttcata gaatccggga agcgtatctg 2940
 ccagagttgt ctgtgattcc aggtgtcaat gcggccattt tcgaagaatt agagggacgt 3000
 atttttacag cgtattcctt atatgatgcg agaaatgtca ttaaaaatgg cgatttcaat 3060
 aatggcttat tatgctggaa cgtgaaagggt catgtagatg tagaagagca aaacaaccac 3120
 35 cggtcgggtc ttgttatccc agaatgggag gcagaagtgt cacaagaggt tcgtgtctgt 3180
 ccaggtcgtg gctatatcct tcgtgtcaca gcatataaag agggatatgg agagggctgc 3240
 gtaacgatcc atgagatcga agacaatata gacgaactga aattcagcaa ctgtgtagaa 3300
 gaggaagtat atccaaacaa cacagtaacg tgtaataatt atactgggac tcaagaagaa 3360
 40 tatgagggta cgtacacttc tcgtaataaa ggatatgacg aagcctatgg taataaccct 3420
 tccgtaccag ctgattacgc ttcagtctat gaagaaaaat cgtatacaga tggacgaaga 3480
 gagaatcctt gtgaatctaa cagaggctat ggggattaca caccactacc ggctggttat 3540
 gtaacaaagg attttagagta ctcccagag accgataaag tatggattga gatcggagaa 3600
 acagaaggaa cattcatcgt ggatagcgtg gaattactcc ttatggagga a 3651

<210> 2

<211> 1217

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: protein
 encoded by the gene SN15

EP 1 099 760 A1

<400> 2

5	Met Lys Leu Lys Asn Gln Asp Lys His Gln Ser Phe Ser Ser Asn Ala	1	5	10	15
	Lys Val Asp Lys Ile Ser Thr Asp Ser Leu Lys Asn Glu Thr Asp Ile	20	25	30	
10	Glu Leu Gln Asn Ile Asn His Glu Asp Cys Leu Lys Met Ser Glu Tyr	35	40	45	
15	Glu Asn Val Glu Pro Phe Val Ser Ala Ser Thr Ile Gln Thr Gly Ile	50	55	60	
	Gly Ile Ala Gly Lys Ile Leu Gly Thr Leu Gly Val Pro Phe Ala Gly	65	70	75	80
20	Gln Val Ala Ser Leu Tyr Ser Phe Ile Leu Gly Glu Leu Trp Pro Lys	85	90	95	
25	Gly Lys Asn Gln Trp Glu Ile Phe Met Glu His Val Glu Glu Ile Ile	100	105	110	
30	Asn Gln Lys Ile Ser Thr Tyr Ala Arg Asn Lys Ala Leu Thr Asp Leu	115	120	125	
	Lys Gly Leu Gly Asp Ala Leu Ala Val Tyr His Asp Ser Leu Glu Ser	130	135	140	
35	Trp Val Gly Asn Arg Asn Asn Thr Arg Ala Arg Ser Val Val Lys Ser	145	150	155	160
40	Gln Tyr Ile Ala Leu Glu Leu Met Phe Val Gln Lys Leu Pro Ser Phe	165	170	175	
	Ala Val Ser Gly Glu Glu Val Pro Leu Leu Pro Ile Tyr Ala Gln Ala	180	185	190	
45	Ala Asn Leu His Leu Leu Leu Leu Arg Asp Ala Ser Ile Phe Gly Lys	195	200	205	
50	Glu Trp Gly Leu Ser Ser Ser Glu Ile Ser Thr Phe Tyr Asn Arg Gln	210	215	220	
	Val Glu Arg Ala Gly Asp Tyr Ser Tyr His Cys Val Lys Trp Tyr Ser	225	230	235	240
55	Thr Gly Leu Asn Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Val Arg				

EP 1 099 760 A1

	245	250	255
5	Tyr Asn Gln Phe Arg Arg Asp Met Thr Leu Met Val Leu Asp Leu Val 260 265 270		
10	Ala Leu Phe Pro Ser Tyr Asp Thr Gln Met Tyr Pro Ile Lys Thr Thr 275 280 285		
	Ala Gln Leu Thr Arg Glu Val Tyr Thr Asp Ala Ile Gly Thr Val His 290 295 300		
15	Pro His Pro Ser Phe Thr Ser Thr Thr Trp Tyr Asn Asn Asn Ala Pro 305 310 315 320		
20	Ser Phe Ser Ala Ile Glu Ala Ala Val Val Arg Asn Pro His Leu Leu 325 330 335		
	Asp Phe Leu Glu Gln Val Thr Ile Tyr Ser Leu Leu Ser Arg Trp Ser 340 345 350		
25	Asn Thr Gln Tyr Met Asn Met Trp Gly Gly His Lys Leu Glu Phe Arg 355 360 365		
30	Thr Ile Gly Gly Thr Leu Asn Ile Ser Thr Gln Gly Ser Thr Asn Thr 370 375 380		
	Ser Ile Asn Pro Val Thr Leu Pro Phe Thr Ser Arg Asp Val Tyr Arg 385 390 395 400		
35	Thr Glu Ser Leu Ala Gly Leu Asn Leu Phe Leu Thr Gln Pro Val Asn 405 410 415		
40	Gly Val Pro Arg Val Asp Phe His Trp Lys Phe Val Thr His Pro Ile 420 425 430		
	Ala Ser Asp Asn Phe Tyr Tyr Pro Gly Tyr Ala Gly Ile Gly Thr Gln 435 440 445		
45	Leu Gln Asp Ser Glu Asn Glu Leu Pro Pro Glu Ala Thr Gly Gln Pro 450 455 460		
50	Asn Tyr Glu Ser Tyr Ser His Arg Leu Ser His Ile Gly Leu Ile Ser 465 470 475 480		
	Ala Ser His Val Lys Ala Ser Val Tyr Ser Trp Thr His Arg Ser Ala 485 490 495		
55	Asp Arg Thr Asn Thr Ile Gly Pro Asn Arg Ile Thr Gln Ile Pro Met		

EP 1 099 760 A1

	500	505	510
5	Val Lys Ala Ser Glu Leu Pro Gln Gly Thr Thr Val Val Arg Gly Pro 515 520 525		
10	Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Asn Thr Gly Gly Phe 530 535 540		
15	Gly Pro Ile Arg Val Thr Val Asn Gly Pro Leu Thr Gln Arg Tyr Arg 545 550 555 560		
20	Ile Gly Phe Arg Tyr Ala Ser Thr Val Asp Phe Asp Phe Phe Val Ser 565 570 575		
25	Arg Gly Gly Thr Thr Val Asn Asn Phe Arg Phe Leu Arg Thr Met Asn 580 585 590		
30	Ser Gly Asp Glu Leu Lys Tyr Gly Asn Phe Val Arg Arg Ala Phe Thr 595 600 605		
35	Thr Pro Phe Thr Phe Thr Gln Ile Gln Asp Ile Ile Arg Thr Ser Ile 610 615 620		
40	Gln Gly Leu Ser Gly Asn Gly Glu Val Tyr Ile Asp Lys Ile Glu Ile 625 630 635 640		
45	Ile Pro Val Thr Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Arg Ala 645 650 655		
50	Gln Glu Ala Val Asn Ala Leu Phe Thr Asn Thr Asn Pro Arg Arg Leu 660 665 670		
55	Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val 675 680 685		
60	Asp Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser 690 695 700		
65	Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu 705 710 715 720		
70	Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg Gly Trp 725 730 735		
75	Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys 740 745 750		
80	Glu Asn Tyr Val Thr Leu Pro Gly Thr Val Asp Glu Cys Tyr Pro Thr		

EP 1 099 760 A1

	755	760	765
5	Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Tyr Thr Arg 770 775 780		
10	Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr 785 790 795 800		
	Leu Ile Arg Tyr Asn Ala Lys His Glu Ile Val Asn Val Pro Gly Thr 805 810 815		
15	Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys Cys Gly 820 825 830		
20	Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp Asn Pro Asp Leu Asp 835 840 845		
	Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe 850 855 860		
25	Thr Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly 865 870 875 880		
30	Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu 885 890 895		
	Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu 900 905 910		
35	Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys 915 920 925		
40	Leu Gln Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu Ser Val 930 935 940		
	Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Val Asp Thr 945 950 955 960		
45	Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val His Arg Ile Arg 965 970 975		
50	Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala 980 985 990		
	Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Tyr Ser Leu Tyr 995 1000 1005		
55	Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Leu		

EP 1 099 760 A1

	1010	1015	1020
5	Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His 1025	1030	1035 1040
10	Arg Ser Val Leu Val Ile Pro Glu Trp Glu Ala Glu Val Ser Gln Glu 1045	1050	1055
15	Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr 1060	1065	1070
20	Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asp 1075	1080	1085
25	Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu Glu Val Tyr 1090	1095	1100
30	Pro Asn Asn Thr Val Thr Cys Asn Asn Tyr Thr Gly Thr Gln Glu Glu 1105	1110	1115 1120
35	Tyr Glu Gly Thr Tyr Thr Ser Arg Asn Gln Gly Tyr Asp Glu Ala Tyr 1125	1130	1135
40	Gly Asn Asn Pro Ser Val Pro Ala Asp Tyr Ala Ser Val Tyr Glu Glu 1140	1145	1150
45	Lys Ser Tyr Thr Asp Gly Arg Arg Glu Asn Pro Cys Glu Ser Asn Arg 1155	1160	1165
50	Gly Tyr Gly Asp Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Asp 1170	1175	1180
55	Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu 1185	1190	1195 1200
	Thr Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu 1205	1210	1215
	Glu		
	<210> 3		
	<211> 3624		
	<212> DNA		
	<213> Artificial Sequence		

EP 1 099 760 A1

<220>

<223> Description of Artificial Sequence: hybrid
protoxin gene SN19

<400> 3

```

atggcttcaa ataggaaaaa tgagaatgaa attataaatg ctgtatcgaa tcattccgca 60
caaatggatc tattaccaga tgctcgtatt gaggatagct tgtgtatagc cgaggggaac 120
10 aatatcgatc catttggttag cgcatcaaca gtccaaacgg gtattaacat agctggtaga 180
atactaggcg tattgggcgt accgtttgct ggacaactag ctagttttta tagttttctt 240
gttggtgaaat tatggccccg cggcagagat cagtgggaaa ttttcctaga acatgtcgaa 300
caacttataa atcaacaaat aacagaaaat gctaggaata cggctcttgc tcgattacaa 360
15 gggttaggag attccttcag agcctatcaa cagtcacttg aagattggct agaaaaaccgt 420
gatgatgcaa gaacgagaag tggtctttat acccaatata tagctttaga acttgatttt 480
cttaatgcga tgccgctttt cgcaattaga aaccaagaag ttccattatt gatggtatat 540
gctcaagctg caaatttaca cctattatta ttgagagatg cctctctttt tggtagtgaa 600
20 tttgggctta catcgagga aattcaacgc tattatgagc gccaaagtga acgaacgaga 660
gattattccg actattgcgt agaatggtat aatacaggtc taaatagctt gagagggaca 720
aatgccgcaa gttgggtacg gtataatcaa ttccgtagag atctaactgt aggagtatta 780
gatctagtgg cactattccc aagctatgac actcgcactt atccaataaa tacgagtgtc 840
cagttaacaa gagaagttta tacagacgca attgggacag tacatccgca tccaagtttt 900
25 acaagtacga cttggtataa taataatgca ccttcgttct ctgccataga ggctgctgtt 960
gttcgaaacc cgcatctact cgattttcta gaacaagtta caatttacag cttattaagt 1020
cgatggagta aacttcagta tatgaatatg tggggaggac ataaactaga attccgaaca 1080
ataggaggaa cgtaaatat ctcaacacaa ggatctacta atacttctat taactctgta 1140
30 acattaccgt tcacttctcg agacgtctat aggactgaat cattggcagg gctgaatcta 1200
ttttaactc aaactgttaa tggagtacct aggggttgatt ttcatggaa attcgtcaca 1260
catccgatcg catctgataa tttctattat ccagggtatg ctggaatttg gacgcaatta 1320
caggattcag aaaaatgaatt accacctgaa gcaacaggac agccaaatta tgaatcttat 1380
35 agtcatagat tatctcatat aggactcatt tcagcatcac atgtgaaaagc atcggtatat 1440
tcttgagcgc atcgtagtgc ggaccgtacg aatacgtatt gaccaaatag aatcacccaa 1500
atccaatgg taaaagcatc cgaacttctt caaggtagca ctgttgtagg aggaccagga 1560
tttactggtg gggatattct tcgaagaacg aatactggtg gatttggacc gataagagta 1620
actgttaacg gaccattaac acaaagatat cgtataggat tccgctatgc ttcaactgta 1680
40 gattttgatt tctttgtatc acgtggaggt actactgtaa ataatttttag attcctacgt 1740
acaatgaaca gtggagacga actaaaatac ggaaattttg tgagacgtgc ttttactaca 1800
ccttttactt ttacacaaat tcaagatata attcgaacgt ctattcaagg ccttagtgga 1860
aatggggaag tgtatataga taaaattgaa attattccag ttactgcaac cttcgaagca 1920
45 gaatatgatt tagaaagagc gcaagaggcg gtgaatgctc tgtttactaa tacgaatcca 1980
agaagattga aaacagatgt gacagattat catattgata aagtatccaa tttagtggat 2040
tgtttatcag atgaattttg tctggatgaa aagcgagaat tgtccgagaa agtcaaacat 2100
gcgaagcgac tcagtgatga gcggaattta cttcaagatc caaacttcag agggatcaat 2160
agacaaccag accgtggctg gagaggaagt acagatatta ccatccaagg aggagatgac 2220
50 gtattcaaag agaattacgt cacactaccg ggtaccgttg atgagtgtca tccaacgcat 2280
ttatatcaga aaatagatga gtcgaaatta aaagcttata cccgttatga attaagaggg 2340
tatatcgaag atagtcaaga cttagaaatc tatttgatcc gttacaatgc aaaacacgaa 2400
atagtaaattg tgccaggcac ggggttcctta tggccgcttt cagcccaaag tccaatcgga 2460
55 aagtgtggag aaccgaatcg atgcgcgcca caccttgaat ggaatcctga tctagattgt 2520
tcctgcagag acggggaaaa atgtgcacat cattcccatc atttcacctt ggatattgat 2580

```


EP 1 099 760 A1

gttgatgta cagacttaaa tgaggactta ggtgtatggg tgatattcaa gattaagacg 2640
 caagatggcc atgcaagact agggaatcta gagtttctcg aagagaaacc attattaggg 2700
 5 gaagcactag ctctgttgaa aagagcggag aagaagtgga gagacaaacg agagaaactg 2760
 cagttggaaa caaatattgt ttataaagag gcaaaagaat ctgtagatgc tttatttgta 2820
 aactctcaat atgatagatt acaagtggat acgaacatcg cgatgattca tgcggcagat 2880
 aaacgcgttc atagaatccg ggaagcgtat ctgccagagt tgtctgtgat tccagggtgc 2940
 10 aatgcggcca ttttcgaaga attagaggga cgtattttta cagcgtattc cttatatgat 3000
 gcgagaaatg tcattaaaaa tggcgatttc aataatggct tattatgctg gaacgtgaaa 3060
 ggtcatgtag atgtagaaga gcaaaacaac caccgttcgg tccttggtat cccagaatgg 3120
 gaggcagaag tgtcacaaga ggttcgtgtc tgtccaggtc gtggctatat ccttcgtgtc 3180
 acagcatata aagagggata tggagagggc tgcgtaacga tccatgagat cgaagacaat 3240
 15 acagacgaac tgaaattcag caactgtgta gaagaggaag tataatccaa caacacagta 3300
 acgtgtaata attatactgg gactcaagaa gaatatgagg gtacgtacac ttctcgtaat 3360
 caaggatatg acgaagccta tggtaataac ccttcgttac cagctgatta cgcttcagtc 3420
 tatgaagaaa aatcgtatac agatggacga agagagaatc cttgtgaatc taacagaggc 3480
 20 tatggggatt acacaccact accggctggt tatgtaacaa aggatttaga gtacttccca 3540
 gagaccgata aggtatggat tgagatcgga gaaacagaag gaacattcat cgtggatagc 3600
 gtggaattac tccttatgga ggaa 3624

25 <210> 4
 <211> 1208
 <212> PRT
 <213> Artificial Sequence
 30
 <220>
 <223> Description of Artificial Sequence: protein
 encoded by the gene SN19
 35

<400> 4
 Met Ala Ser Asn Arg Lys Asn Glu Asn Glu Ile Ile Asn Ala Val Ser
 1 5 10 15
 40 Asn His Ser Ala Gln Met Asp Leu Leu Pro Asp Ala Arg Ile Glu Asp
 20 25 30
 45 Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp Pro Phe Val Ser Ala
 35 40 45
 Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly Arg Ile Leu Gly Val
 50 55 60
 50 Leu Gly Val Pro Phe Ala Gly Gln Leu Ala Ser Phe Tyr Ser Phe Leu
 65 70 75 80
 55 Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Gln Trp Glu Ile Phe Leu
 85 90 95

EP 1 099 760 A1

	Glu His Val Glu Gln Leu Ile Asn Gln Gln Ile Thr Glu Asn Ala Arg	
	100	105 110
5	Asn Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly Asp Ser Phe Arg Ala	
	115	120 125
10	Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn Arg Asp Asp Ala Arg	
	130	135 140
	Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala Leu Glu Leu Asp Phe	
15	145	150 155 160
	Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn Gln Glu Val Pro Leu	
	165	170 175
20	Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Arg	
	180	185 190
	Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu Thr Ser Gln Glu Ile	
25	195	200 205
	Gln Arg Tyr Tyr Glu Arg Gln Val Glu Arg Thr Arg Asp Tyr Ser Asp	
	210	215 220
30	Tyr Cys Val Glu Trp Tyr Asn Thr Gly Leu Asn Ser Leu Arg Gly Thr	
	225	230 235 240
	Asn Ala Ala Ser Trp Val Arg Tyr Asn Gln Phe Arg Arg Asp Leu Thr	
35	245	250 255
	Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro Ser Tyr Asp Thr Arg	
	260	265 270
40	Thr Tyr Pro Ile Asn Thr Ser Ala Gln Leu Thr Arg Glu Val Tyr Thr	
	275	280 285
45	Asp Ala Ile Gly Thr Val His Pro His Pro Ser Phe Thr Ser Thr Thr	
	290	295 300
	Trp Tyr Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu Ala Ala Val	
50	305	310 315 320
	Val Arg Asn Pro His Leu Leu Asp Phe Leu Glu Gln Val Thr Ile Tyr	
	325	330 335
55	Ser Leu Leu Ser Arg Trp Ser Asn Thr Gln Tyr Met Asn Met Trp Gly	
	340	345 350

EP 1 099 760 A1

Gly His Lys Leu Glu Phe Arg Thr Ile Gly Gly Thr Leu Asn Ile Ser
 355 360 365
 5 Thr Gln Gly Ser Thr Asn Thr Ser Ile Asn Pro Val Thr Leu Pro Phe
 370 375 380
 10 Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Leu Ala Gly Leu Asn Leu
 385 390 395 400
 Phe Leu Thr Gln Pro Val Asn Gly Val Pro Arg Val Asp Phe His Trp
 405 410 415
 15 Lys Phe Val Thr His Pro Ile Ala Ser Asp Asn Phe Tyr Tyr Pro Gly
 420 425 430
 20 Tyr Ala Gly Ile Gly Thr Gln Leu Gln Asp Ser Glu Asn Glu Leu Pro
 435 440 445
 Pro Glu Ala Thr Gly Gln Pro Asn Tyr Glu Ser Tyr Ser His Arg Leu
 450 455 460
 25 Ser His Ile Gly Leu Ile Ser Ala Ser His Val Lys Ala Ser Val Tyr
 465 470 475 480
 30 Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn Thr Ile Gly Pro Asn
 485 490 495
 Arg Ile Thr Gln Ile Pro Met Val Lys Ala Ser Glu Leu Pro Gln Gly
 500 505 510
 35 Thr Thr Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg
 515 520 525
 40 Arg Thr Asn Thr Gly Gly Phe Gly Pro Ile Arg Val Thr Val Asn Gly
 530 535 540
 Pro Leu Thr Gln Arg Tyr Arg Ile Gly Phe Arg Tyr Ala Ser Thr Val
 545 550 555 560
 Asp Phe Asp Phe Phe Val Ser Arg Gly Gly Thr Thr Val Asn Asn Phe
 565 570 575
 50 Arg Phe Leu Arg Thr Met Asn Ser Gly Asp Glu Leu Lys Tyr Gly Asn
 580 585 590
 55 Phe Val Arg Arg Ala Phe Thr Thr Pro Phe Thr Phe Thr Gln Ile Gln
 595 600 605

EP 1 099 760 A1

	Asp Ile Ile Arg Thr Ser Ile Gln Gly Leu Ser Gly Asn Gly Glu Val	
	610	615 620
5	Tyr Ile Asp Lys Ile Glu Ile Ile Pro Val Thr Ala Thr Phe Glu Ala	
	625	630 635 640
10	Glu Tyr Asp Leu Glu Arg Ala Gln Glu Ala Val Asn Ala Leu Phe Thr	
	645	650 655
15	Asn Thr Asn Pro Arg Arg Leu Lys Thr Asp Val Thr Asp Tyr His Ile	
	660	665 670
20	Asp Gln Val Ser Asn Leu Val Asp Cys Leu Ser Asp Glu Phe Cys Leu	
	675	680 685
25	Asp Glu Lys Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu	
	690	695 700
30	Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile Asn	
	705	710 715 720
35	Arg Gln Pro Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln	
	725	730 735
40	Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly Thr	
	740	745 750
45	Val Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser	
	755	760 765
50	Lys Leu Lys Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu Asp	
	770	775 780
55	Ser Gln Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu	
	785	790 795 800
60	Ile Val Asn Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Ala Gln	
	805	810 815
65	Ser Pro Ile Gly Lys Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu	
	820	825 830
70	Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys Cys	
	835	840 845
75	Ala His His Ser His His Phe Thr Leu Asp Ile Asp Val Gly Cys Thr	
	850	855 860

EP 1 099 760 A1

	Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr	
	865	870 875 880
5	Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys	
		885 890 895
10	Pro Leu Leu Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys	
		900 905 910
	Trp Arg Asp Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val Tyr	
		915 920 925
15	Lys Glu Ala Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr	
		930 935 940
20	Asp Arg Leu Gln Val Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp	
		945 950 955 960
	Lys Arg Val His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val	
		965 970 975
25	Ile Pro Gly Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile	
		980 985 990
30	Phe Thr Ala Tyr Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly	
		995 1000 1005
	Asp Phe Asn Asn Gly Leu Leu Cys Trp Asn Val Lys Gly His Val Asp	
35		1010 1015 1020
	Val Glu Glu Gln Asn Asn His Arg Ser Val Leu Val Ile Pro Glu Trp	
		1025 1030 1035 1040
40	Glu Ala Glu Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr	
		1045 1050 1055
	Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val	
45		1060 1065 1070
	Thr Ile His Glu Ile Glu Asp Asn Thr Asp Glu Leu Lys Phe Ser Asn	
		1075 1080 1085
50	Cys Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn Asn	
		1090 1095 1100
55	Tyr Thr Gly Thr Gln Glu Glu Tyr Glu Gly Thr Tyr Thr Ser Arg Asn	
		1105 1110 1115 1120

EP 1 099 760 A1

Gln Gly Tyr Asp Glu Ala Tyr Gly Asn Asn Pro Ser Val Pro Ala Asp
1125 1130 1135

5 Tyr Ala Ser Val Tyr Glu Glu Lys Ser Tyr Thr Asp Gly Arg Arg Glu
1140 1145 1150

Asn Pro Cys Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu Pro
10 1155 1160 1165

Ala Gly Tyr Val Thr Lys Asp Leu Glu Tyr Phe Pro Glu Thr Asp Lys
1170 1175 1180

15 Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val Asp Ser
1185 1190 1195 1200

20 Val Glu Leu Leu Leu Met Glu Glu
1205

25 <210> 5
<211> 3627
<212> DNA
<213> Artificial Sequence

30 <220>
<223> Description of Artificial Sequence: modified
crylBa gene

35 <400> 5
atgacttcaa ataggaaaa tgagaatgaa attataaatg ctgtatcgaa tcattccgca 60
caaatggatc tattaccaga tgctcgtatt gaggatagct tgtgtatagc cgaggggaac 120
aatatcgatc catttggttag cgcatcaaca gtccaaacgg gtattaacat agctggtaga 180
40 atactaggcg tattgggctg accgtttgct ggacaactag ctagttttta tagttttctt 240
gttggtgaat tatggcccg cggcagagat cagtgggaaa ttttcttaga acatgtcgaa 300
caacttataa atcaacaaat aacagaaaat gctaggaata cggtcttgc tcgattacaa 360
ggttaggag attccttcag agcctatcaa cagtcacttg aagattggct agaaaaccgt 420
45 gatgatgcaa gaacgagaag tgttctttat acccaatata tagctttaga acttgatttt 480
cttaatgcga tgccgctttt cgcaattaga aaccaagaag ttccattatt gatggtatat 540
gctcaagctg caaatttaca cctattatta ttgagagatg cctctctttt tggtagtgaa 600
tttgggctta catcgagga aattcaacgc tattatgagc gccaaagtga acgaacgaga 660
gattattccg actattgctg agaatggtat aatacaggct taaatagctt gagagggaca 720
50 aatgcgcgaa gtgggttacg gtataatcaa ctccgtagag atctaacgtt aggagtatta 780
gatctagtgg cactattccc aagctatgac actcgactt atccaataaa tacgagtgc 840
cagttaacaa gagaagtta tacagacgca attggagcaa caggggtaaa tatggcaagt 900
atgaattggt ataataataa tgcaccttcg ttctctgcca tagaggctgc ggctatccga 960
55 agcccgcatc tacttgattt tctagaacaa cttacaattt ttagcgcttc atcacgatgg 1020
agtaatacta ggcataatgac ttattggcgg gggcacacga ttcaatctcg gccaatagga 1080

EP 1 099 760 A1

5 ggcggtattaa atacctcaac gcatggggct accaatactt ctattaatcc tgtaacatta 1140
 cggttcgcat ctcgagacgt ttataggact gaatcatatg caggagtgc tctatgggga 1200
 atttaccttg aacctattca tgggtgccct actggttaggt ttaattttac gaaccctcag 1260
 aatattttctg atagaggtag cgctaactat agtcaacctt atgagtcacc tgggcttcaa 1320
 ttaaagatt cagaaactga attaccacca gaaacaacag aacgaccaa ttatgaatct 1380
 tacagtcaca gggtatctca tataaggata attttacaat ccagggtgaa tgtaccggta 1440
 tattcttgga cgcacgtag tgcggaccgt acgaatacga ttggaccaa tagaatcacc 1500
 10 caaatcccaa tggtaaaagc atccgaactt cctcaaggta cactgttgt tagaggacca 1560
 ggatttactg gtggggatat tcttcgaaga acgaatactg gtggatttg accgataaga 1620
 gtaactgtta acggaccatt aacacaaaga tatcgtag gattccgcta tgcctcaact 1680
 gtagattttg atttctttgt atcacgtgga ggtactactg taaataatt tagattccta 1740
 cgtacaatga acagtggaga cgaactaaaa tacggaaatt ttgtgagacg tgcctttact 1800
 15 acacccttta cttttacaca aattcaagat ataattcgaa cgtctattca aggccttagt 1860
 ggaaatgggg aagtgtatat agataaaatt gaaattattc cagttactgc aaccttcgaa 1920
 gcagaatatg atttagaaag agcgcaagag gcggtgaatg ctctgtttac taatacgaat 1980
 ccaagaagat tgaaaacaga tgtgacagat tatcatattg atcaagtatc caatttagtg 2040
 20 gattgtttat cagatgaatt ttgtctggat gaaaagcgag aattgtccga gaaagtcaaa 2100
 catgcgaagc gactcagtag tgagcggaat ttacttcaag atccaaactt cagagggatc 2160
 aatagacaac cagaccgtgg ctggagagga agtacagata ttaccatcca aggaggagat 2220
 gacgtattca aagagaatta cgtcacacta ccgggtaccg ttgatgagtg ctatccaacg 2280
 25 tatttatatc agaaaataga tgagtcgaaa taaaagctt ataccggtta tgaattaaga 2340
 gggatatatc aagatagtca agacttagaa atctatttga tccgttacaa tgcaaaacac 2400
 gaaatagtaa atgtgccag caccgggttc ttatggccgc tttagccca aagtcacac 2460
 ggaaagtgtg gagaaccgaa tcgatgcgcg ccacacctt aatggaatcc tgatctagat 2520
 tgttcttgca gagacgggga aaaatgtgca catcattccc atcatttcac cttggatatt 2580
 30 gatgttgat gtacagactt aaatgaggac ttatgtgtat ggggtgatatt caagattaag 2640
 acgcaagatg gccatgcaag actagggaat ctatagtttc tcgaagagaa accattatta 2700
 ggggaagcac tagctcgtgt gaaaagagcg gagaagaagt ggagagacaa acgagagaaa 2760
 ctgcagttg aaacaaatat tgtttataaa gaggcaaaag aatctgtaga tgctttattt 2820
 35 gtaaaacttc aatatgatag attacaagt gatacgaaca tcgcatgat tcatgcggca 2880
 gataaacgag ttcatagaat ccgggaagcg tatctgccag agttgtctgt gattccaggt 2940
 gtcaatgcg ccattttcga agaattagag ggacgtattt ttacagcgta ttctttatat 3000
 gatgcgagaa atgtcattaa aaatggcgat ttcaataatg gcttattatg ctggaacgtg 3060
 40 aaaggtcatg tagatgtaga agagcaaaac aaccaccgtt cggctcctgt tatcccagaa 3120
 tgggaggcag aagtgtcaca agaggttcgt gtctgtccag gtcgtggcta tatcctcgt 3180
 gtcacagcat ataaagagg atatggagag ggctgcgtaa ccatccatga gatcgaagac 3240
 aatacagacg aactgaaatt cagcaactgt gtagaagagg aagtatatcc aaacaacaca 3300
 gtaacgtgta ataattatc tgggactcaa gaagaatatg agggtagcta cacttctcgt 3360
 45 aatcaaggat atgacgaagc ctatggtaat aacccttccg taccagctga ttacgcttca 3420
 gtctatgaag aaaaatcgta tacagatgga cgaagagaga atccttgtga atctaacaga 3480
 ggctatgggg attacacacc actaccggct ggttatgtaa caaaggattt agagtacttc 3540
 ccagagaccg ataaggtatg gattgagatc ggagaaacag aaggaacatt catcgtggat 3600
 50 agcgtggaat tactccttat ggaggaa 3627

<210> 6

<211> 1209

<212> PRT

EP 1 099 760 A1

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: protein
encoded by the modified cry1Ba gene

<400> 6

5
10 Met Thr Ser Asn Arg Lys Asn Glu Asn Glu Ile Ile Asn Ala Val Ser
1 5 10 15
15 Asn His Ser Ala Gln Met Asp Leu Leu Pro Asp Ala Arg Ile Glu Asp
20 25 30
20 Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp Pro Phe Val Ser Ala
35 40 45
25 Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly Arg Ile Leu Gly Val
50 55 60
30 Leu Gly Val Pro Phe Ala Gly Gln Leu Ala Ser Phe Tyr Ser Phe Leu
65 70 75 80
35 Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Gln Trp Glu Ile Phe Leu
85 90 95
40 Glu His Val Glu Gln Leu Ile Asn Gln Gln Ile Thr Glu Asn Ala Arg
100 105 110
45 Asn Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly Asp Ser Phe Arg Ala
115 120 125
50 Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn Arg Asp Asp Ala Arg
130 135 140
55 Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala Leu Glu Leu Asp Phe
145 150 155 160
60 Leu Asn-Ala Met Pro Leu Phe Ala Ile Arg Asn Gln Glu Val Pro Leu
165 170 175
65 Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Arg
180 185 190
70 Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu Thr Ser Gln Glu Ile
195 200 205
75 Gln Arg Tyr Tyr Glu Arg Gln Val Glu Arg Thr Arg Asp Tyr Ser Asp
210 215 220

EP 1 099 760 A1

Tyr Cys Val Glu Trp Tyr Asn Thr Gly Leu Asn Ser Leu Arg Gly Thr
 225 230 235 240
 5 Asn Ala Ala Ser Trp Val Arg Tyr Asn Gln Phe Arg Arg Asp Leu Thr
 245 250 255
 10 Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro Ser Tyr Asp Thr Arg
 260 265 270
 Thr Tyr Pro Ile Asn Thr Ser Ala Gln Leu Thr Arg Glu Val Tyr Thr
 275 280 285
 15 Asp Ala Ile Gly Ala Thr Gly Val Asn Met Ala Ser Met Asn Trp Tyr
 290 295 300
 20 Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu Ala Ala Ala Ile Arg
 305 310 315 320
 Ser Pro His Leu Leu Asp Phe Leu Glu Gln Leu Thr Ile Phe Ser Ala
 325 330 335
 25 Ser Ser Arg Trp Ser Asn Thr Arg His Met Thr Tyr Trp Arg Gly His
 340 345 350
 30 Thr Ile Gln Ser Arg Pro Ile Gly Gly Gly Leu Asn Thr Ser Thr His
 355 360 365
 Gly Ala Thr Asn Thr Ser Ile Asn Pro Val Thr Leu Arg Phe Ala Ser
 370 375 380
 35 Arg Asp Val Tyr Arg Thr Glu Ser Tyr Ala Gly Val Leu Leu Trp Gly
 385 390 395 400
 40 Ile Tyr Leu Glu Pro Ile His Gly Val Pro Thr Val Arg Phe Asn Phe
 405 410 415
 Thr Asn Pro Gln Asn Ile Ser Asp Arg Gly Thr Ala Asn Tyr Ser Gln
 420 425 430
 Pro Tyr Glu Ser Pro Gly Leu Gln Leu Lys Asp Ser Glu Thr Glu Leu
 435 440 445
 50 Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser Tyr Ser His Arg
 450 455 460
 55 Leu Ser His Ile Gly Ile Ile Leu Gln Ser Arg Val Asn Val Pro Val
 465 470 475 480

EP 1 099 760 A1

Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn Thr Ile Gly Pro
 485 490 495
 5
 Asn Arg Ile Thr Gln Ile Pro Met Val Lys Ala Ser Glu Leu Pro Gln
 500 505 510
 10
 Gly Thr Thr Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu
 515 520 525
 Arg Arg Thr Asn Thr Gly Gly Phe Gly Pro Ile Arg Val Thr Val Asn
 530 535 540
 15
 Gly Pro Leu Thr Gln Arg Tyr Arg Ile Gly Phe Arg Tyr Ala Ser Thr
 545 550 555 560
 20
 Val Asp Phe Asp Phe Phe Val Ser Arg Gly Gly Thr Thr Val Asn Asn
 565 570 575
 Phe Arg Phe Leu Arg Thr Met Asn Ser Gly Asp Glu Leu Lys Tyr Gly
 580 585 590
 25
 Asn Phe Val Arg Arg Ala Phe Thr Thr Pro Phe Thr Phe Thr Gln Ile
 595 600 605
 30
 Gln Asp Ile Ile Arg Thr Ser Ile Gln Gly Leu Ser Gly Asn Gly Glu
 610 615 620
 Val Tyr Ile Asp Lys Ile Glu Ile Ile Pro Val Thr Ala Thr Phe Glu
 625 630 635 640
 35
 Ala Glu Tyr Asp Leu Glu Arg Ala Gln Glu Ala Val Asn Ala Leu Phe
 645 650 655
 40
 Thr Asn Thr Asn Pro Arg Arg Leu Lys Thr Asp Val Thr Asp Tyr His
 660 665 670
 Ile Asp Gln Val Ser Asn Leu Val Asp Cys Leu Ser Asp Glu Phe Cys
 675 680 685
 45
 Leu Asp Glu Lys Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg
 690 695 700
 50
 Leu Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile
 705 710 715 720
 55
 Asn Arg Gln Pro Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile
 725 730 735

EP 1 099 760 A1

5 Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly
 740 745 750
 Thr Val Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu
 755 760 765
 10 Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu
 770 775 780
 Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His
 785 790 795 800
 15 Glu Ile Val Asn Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Ala
 805 810 815
 20 Gln Ser Pro Ile Gly Lys Cys Gly Glu Pro Asn Arg Cys Ala Pro His
 820 825 830
 Leu Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys
 25 835 840 845
 Cys Ala His His Ser His His Phe Thr Leu Asp Ile Asp Val Gly Cys
 850 855 860
 30 Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys
 865 870 875 880
 Thr Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu
 35 885 890 895
 Lys Pro Leu Leu Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys
 900 905 910
 40 Lys Trp Arg Asp Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val
 915 920 925
 Tyr Lys Glu Ala Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln
 45 930 935 940
 Tyr Asp Arg Leu Gln Val Asp Thr Asn Ile Ala Met Ile His Ala Ala
 945 950 955 960
 50 Asp Lys Arg Val His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser
 965 970 975
 Val Ile Pro Gly Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg
 55 980 985 990

EP 1 099 760 A1

Ile Phe Thr Ala Tyr Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn
995 1000 1005

5 Gly Asp Phe Asn Asn Gly Leu Leu Cys Trp Asn Val Lys Gly His Val
1010 1015 1020

10 Asp Val Glu Glu Gln Asn Asn His Arg Ser Val Leu Val Ile Pro Glu
1025 1030 1035 1040

Trp Glu Ala Glu Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly
1045 1050 1055

15 Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys
1060 1065 1070

20 Val Thr Ile His Glu Ile Glu Asp Asn Thr Asp Glu Leu Lys Phe Ser
1075 1080 1085

Asn Cys Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn
1090 1095 1100

25 Asn Tyr Thr Gly Thr Gln Glu Glu Tyr Glu Gly Thr Tyr Thr Ser Arg
1105 1110 1115 1120

30 Asn Gln Gly Tyr Asp Glu Ala Tyr Gly Asn Asn Pro Ser Val Pro Ala
1125 1130 1135

Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ser Tyr Thr Asp Gly Arg Arg
1140 1145 1150

35 Glu Asn Pro Cys Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu
1155 1160 1165

40 Pro Ala Gly Tyr Val Thr Lys Asp Leu Glu Tyr Phe Pro Glu Thr Asp
1170 1175 1180

45 ~~Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val Asp~~
1185 1190 1195 1200

Ser Val Glu Leu Leu Leu Met Glu Glu
1205

50

<210> 7
55 <211> 2160
<212> DNA

EP 1 099 760 A1

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: modified
cryIIa gene

<400> 7

```

10 atgaaactaa agaatcaaga taagcatcaa agtttttcta gcaatgcgaa agtagataaa 60
   atctctacgg attcactaaa aaatgaaaca gatatagaat tacaaaacat taatcatgaa 120
   gattgtttga aaatgtctga gtatgaaaat gtagagccgt ttgttagtgc atcaacaatt 180
   caaacaggta ttggtattgc gggtaaaata cttgggtaccc taggcgttcc ttttgcagga 240
15 caagtagcta gtctttatag ttttatctta ggtgagctat ggccctaaggg gaaaaatcaa 300
   tgggaaatct ttatggaaca tgtagaagag attattaatc aaaaaatatac aacttatgca 360
   agaaataaag cacttacaga cttgaaagga ttaggagatg ccttagctgt ctacatgat 420
   tcgcttgaaa gttgggttgg aaatcgtaat aacacaaggg ctaggagtgt tgtcaagagc 480
   caatatatcg cattagaatt gatgttcggt cagaaactac cttcttttgc agtgtctgga 540
20 gaggaggtac cattattacc gatatatgcc caagctgcaa atttacattt gttgctatta 600
   agagatgcat ctattttttg aaaagagtgg ggattatcat cttcagaaat ttcaacattt 660
   tataaccgtc aagtcgaacg agcaggagat tattcctacc attgtgtgaa atgggtatagc 720
   acaggtctaa ataacttgag gggtaaaaat gccgaaagtt gggtagcata taatcaattc 780
25 cgtagagaca tgactttaat ggtactagat ttagtggcac tatttccaag ctatgatata 840
   caaatgtatc caattaaaac tacagcccaa cttacaagag aagtatatac agacgcaatt 900
   gggacagtac atccgcatcc aagttttaca agtacgactt ggtataataa taatgcacct 960
   tcgttctctg ccatagaggc tgctgttgtt cgaaaccgc atctactcga ttttctagaa 1020
30 caagttacaa ttacagctt attaagtcga tggagtaaca ctcagtatat gaatatgtgg 1080
   ggaggacata aactagaatt ccgaacaata ggaggaacgt taaatatctc aacacaagga 1140
   tctactaata cttctattaa tcctgtaaca ttaccgttca cttctcgaga cgtctatagg 1200
   actgaatcat tggcagggct gaatctatct ttaactcaac ctgttaatgg agtacctagg 1260
   gttgattttc attggaaatt cgtcacacat ccgacgcac ctgataattt ctattatcca 1320
35 gggtagtctg gaattgggac gcaattacag gattcagaaa atgaattacc acctgaagca 1380
   acaggacagc caaattatga atcttatagt catagattat ctcatatagg actcatttca 1440
   gcatcacatg tgaagcatt ggtatattct tggacgcac gtagtgcgga ccgtacaaat 1500
   acaattgagc caaatagcat tacacaaata ccattagtaa aagctttcaa tctgtcttca 1560
40 ggtgccgctg tagtgagagg accaggattt acaggtgggg atatccttcg aagaacgaat 1620
   actggtacat ttggggatat acgagtaaat attaatccac catttgaca aagatatcgc 1680
   gtgaggattc gctatgcttc taccacagat ttacaattcc atacgtcaat taacggtaaa 1740
   gctattaatc aaggtaattt ttcagcaact atgaatagag gagaggactt agactataaa 1800
45 acctttagaa ctgtaggctt taccactcca tttagctttt tagatgtaca aagtacattc 1860
   acaataggtg cttggaactt ctcttcaggt aacgaagttt atatagatag aattgaattt 1920
   gttccggtag aagtaacata tgaggcagaa tatgattttg aaaaagcgca agagaagggt 1980
   actgcactgt ttacatctac gaatccaaga ggattaaaaa cagatgtaaa ggattatcat 2040
   attgaccagg tatcaaatat agtagagtct ctatcagatg aattctatct tgatgaaaag 2100
50 agagaattat tcgagatagt taaatacgcg aagcaactcc atattgagcg taacatgtag 2160

```

<210> 8

<211> 719

<212> PRT

EP 1 099 760 A1

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: protein
encoded by the modified cryIIa gene

<400> 8

Met Lys Leu Lys Asn Gln Asp Lys His Gln Ser Phe Ser Ser Asn Ala
1 5 10 15

Lys Val Asp Lys Ile Ser Thr Asp Ser Leu Lys Asn Glu Thr Asp Ile
20 25 30

Glu Leu Gln Asn Ile Asn His Glu Asp Cys Leu Lys Met Ser Glu Tyr
35 40 45

Glu Asn Val Glu Pro Phe Val Ser Ala Ser Thr Ile Gln Thr Gly Ile
50 55 60

Gly Ile Ala Gly Lys Ile Leu Gly Thr Leu Gly Val Pro Phe Ala Gly
65 70 75 80

Gln Val Ala Ser Leu Tyr Ser Phe Ile Leu Gly Glu Leu Trp Pro Lys
85 90 95

Gly Lys Asn Gln Trp Glu Ile Phe Met Glu His Val Glu Glu Ile Ile
100 105 110

Asn Gln Lys Ile Ser Thr Tyr Ala Arg Asn Lys Ala Leu Thr Asp Leu
115 120 125

Lys Gly Leu Gly Asp Ala Leu Ala Val Tyr His Asp Ser Leu Glu Ser
130 135 140

Trp Val Gly Asn Arg Asn Asn Thr Arg Ala Arg Ser Val Val Lys Ser
145 150 155 160

Gln Tyr Ile Ala Leu Glu Leu Met Phe Val Gln Lys Leu Pro Ser Phe
165 170 175

Ala Val Ser Gly Glu Glu Val Pro Leu Leu Pro Ile Tyr Ala Gln Ala
180 185 190

Ala Asn Leu His Leu Leu Leu Leu Arg Asp Ala Ser Ile Phe Gly Lys
195 200 205

Glu Trp Gly Leu Ser Ser Ser Glu Ile Ser Thr Phe Tyr Asn Arg Gln
210 215 220

EP 1 099 760 A1

	Val	Glu	Arg	Ala	Gly	Asp	Tyr	Ser	Tyr	His	Cys	Val	Lys	Trp	Tyr	Ser	225	230	235	240
5	Thr	Gly	Leu	Asn	Asn	Leu	Arg	Gly	Thr	Asn	Ala	Glu	Ser	Trp	Val	Arg	245	250	255	
10	Tyr	Asn	Gln	Phe	Arg	Arg	Asp	Met	Thr	Leu	Met	Val	Leu	Asp	Leu	Val	260	265	270	
15	Ala	Leu	Phe	Pro	Ser	Tyr	Asp	Thr	Gln	Met	Tyr	Pro	Ile	Lys	Thr	Thr	275	280	285	
	Ala	Gln	Leu	Thr	Arg	Glu	Val	Tyr	Thr	Asp	Ala	Ile	Gly	Thr	Val	His	290	295	300	
20	Pro	His	Pro	Ser	Phe	Thr	Ser	Thr	Thr	Trp	Tyr	Asn	Asn	Asn	Ala	Pro	305	310	315	320
25	Ser	Phe	Ser	Ala	Ile	Glu	Ala	Ala	Val	Val	Arg	Asn	Pro	His	Leu	Leu	325	330	335	
	Asp	Phe	Leu	Glu	Gln	Val	Thr	Ile	Tyr	Ser	Leu	Leu	Ser	Arg	Trp	Ser	340	345	350	
30	Asn	Thr	Gln	Tyr	Met	Asn	Met	Trp	Gly	Gly	His	Lys	Leu	Glu	Phe	Arg	355	360	365	
35	Thr	Ile	Gly	Gly	Thr	Leu	Asn	Ile	Ser	Thr	Gln	Gly	Ser	Thr	Asn	Thr	370	375	380	
40	Ser	Ile	Asn	Pro	Val	Thr	Leu	Pro	Phe	Thr	Ser	Arg	Asp	Val	Tyr	Arg	385	390	395	400
	Thr	Glu	Ser	Leu	Ala	Gly	Leu	Asn	Leu	Phe	Leu	Thr	Gln	Pro	Val	Asn	405	410	415	
45	Gly	Val	Pro	Arg	Val	Asp	Phe	His	Trp	Lys	Phe	Val	Thr	His	Pro	Ile	420	425	430	
50	Ala	Ser	Asp	Asn	Phe	Tyr	Tyr	Pro	Gly	Tyr	Ala	Gly	Ile	Gly	Thr	Gln	435	440	445	
	Leu	Gln	Asp	Ser	Glu	Asn	Glu	Leu	Pro	Pro	Glu	Ala	Thr	Gly	Gln	Pro	450	455	460	
55	Asn	Tyr	Glu	Ser	Tyr	Ser	His	Arg	Leu	Ser	His	Ile	Gly	Leu	Ile	Ser	465	470	475	480

EP 1 099 760 A1

	Ala	Ser	His	Val	Lys	Ala	Leu	Val	Tyr	Ser	Trp	Thr	His	Arg	Ser	Ala	
					485					490					495		
5	Asp	Arg	Thr	Asn	Thr	Ile	Glu	Pro	Asn	Ser	Ile	Thr	Gln	Ile	Pro	Leu	
				500					505					510			
10	Val	Lys	Ala	Phe	Asn	Leu	Ser	Ser	Gly	Ala	Ala	Val	Val	Arg	Gly	Pro	
			515					520					525				
15	Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Asn	Thr	Gly	Thr	Phe	
		530					535					540					
20	Gly	Asp	Ile	Arg	Val	Asn	Ile	Asn	Pro	Pro	Phe	Ala	Gln	Arg	Tyr	Arg	
	545					550					555					560	
25	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asp	Leu	Gln	Phe	His	Thr	Ser	
					565					570					575		
30	Ile	Asn	Gly	Lys	Ala	Ile	Asn	Gln	Gly	Asn	Phe	Ser	Ala	Thr	Met	Asn	
			580						585					590			
35	Arg	Gly	Glu	Asp	Leu	Asp	Tyr	Lys	Thr	Phe	Arg	Thr	Val	Gly	Phe	Thr	
			595					600					605				
40	Thr	Pro	Phe	Ser	Phe	Leu	Asp	Val	Gln	Ser	Thr	Phe	Thr	Ile	Gly	Ala	
		610					615					620					
45	Trp	Asn	Phe	Ser	Ser	Gly	Asn	Glu	Val	Tyr	Ile	Asp	Arg	Ile	Glu	Phe	
	625					630				635					640		
50	Val	Pro	Val	Glu	Val	Thr	Tyr	Glu	Ala	Glu	Tyr	Asp	Phe	Glu	Lys	Ala	
				645					650					655			
55	Gln	Glu	Lys	Val	Thr	Ala	Leu	Phe	Thr	Ser	Thr	Asn	Pro	Arg	Gly	Leu	
			660					665					670				
60	Lys	Thr	Asp	Val	Lys	Asp	Tyr	His	Ile	Asp	Gln	Val	Ser	Asn	Leu	Val	
		675					680						685				
65	Glu	Ser	Leu	Ser	Asp	Glu	Phe	Tyr	Leu	Asp	Glu	Lys	Arg	Glu	Leu	Phe	
		690				695					700						
70	Glu	Ile	Val	Lys	Tyr	Ala	Lys	Gln	Leu	His	Ile	Glu	Arg	Asn	Met		
	705					710					715						

EP 1 099 760 A1

<210> 9

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: oligomer used
for mutagenesis of the crylBa gene

<400> 9

ggacgcacatcg tagtgccggac cgtacgaata cgattgg

37

<210> 10

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: oligomer used
for mutagenesis of the crylIa gene

<400> 10

ggacgcacatcg tagtgccggac cgtacaaata caattg

36

Claims

1. *Bacillus thuringiensis* hybrid toxin fragment comprising structural domains I, II and III in this order starting from the N-terminal, wherein the domains are derived from at least two different Cry proteins, domain I is domain I of any *Bacillus thuringiensis* Cry protein or a part of said domain or a peptide substantially similar to said domain, domain II is domain II of Cry1Ia or a part of said domain or a peptide substantially similar to said domain, and domain III is domain III of Cry1Ba or a part of said domain or a peptide substantially similar to said domain.
2. Toxin fragment according to claim 1, wherein domain I is domain I of Cry1Ia or Cry1Ba or a part of said domain or a peptide substantially similar to said domain.
3. Toxin fragment according to claim 1 or 2 comprising an amino acid sequence from about amino acid 20 to about amino acid 641 as shown in SEQ ID NO:2 or an amino acid sequence from about amino acid 20 to about amino acid 632 as shown in SEQ ID NO:4.
4. A hybrid toxin comprising the fragment of any one of the preceding claims, or a toxin at least 85% similar to such a hybrid toxin which has substantially similar insecticidal activity.
5. A toxin according to claim 4 comprising an amino acid sequence as shown in SEQ ID NO:2 or SEQ ID NO:4.
6. Pure proteins which are at least 90% identical to the toxin fragments or hybrid toxins of any of claims 1-5.
7. Recombinant DNA comprising a sequence encoding a protein having an amino acid sequence of the proteins as claimed in any one of claims 1-6.
8. Recombinant DNA according to claim 7 comprising the sequence as shown in SEQ ID NO:1 or 3 or DNA similar

thereto encoding a substantially similar protein.

9. Recombinant DNA according to claim 7 comprising the nucleotide sequence from about nucleotide 170 to about 1929 in SEQ ID NO:1 or from about 147 to about 1896 in SEQ ID NO:3.

10. Recombinant DNA according to any of claims 7-9, which further encodes a protein having herbicide resistance, plant growth-promoting, anti-fungal, anti-bacterial, antiviral and/or anti-nematode properties.

11. Recombinant DNA according to any one of claims 7 to 10 which is modified in that known mRNA instability motifs or polyadenylation signals are removed and/or codons which are preferred by the organism into which the recombinant DNA is to be inserted are used so that expression of the thus modified DNA in the said organism yields substantially similar protein to that obtained by expression of the unmodified recombinant DNA in the organism in which the protein components of the hybrid toxin or toxin fragments are endogenous.

12. A DNA sequence which is complementary to one which hybridizes under stringent conditions with the DNA of any one of claims 7 to 11.

13. A vector containing a DNA sequence as claimed in any one of claims 7 to 12.

14. A plant or micro-organism which includes, and enables expression of, the DNA of any one of claims 7-12 or the vector of claim 13.

15. Plants transformed with recombinant DNA as claimed in any one of claims 7 to 12, the progeny of such plants which contain the DNA stably incorporated and heritable in a Mendelian manner, and/or the seeds of such plants and such progeny.

16. Protein derived from expression of the DNA as claimed in any one of claims 7 to 12 and insecticidal protein produced by expression of the recombinant DNA within plants as claimed in claim 15.

17. An insecticidal composition containing one or more of the proteins as claimed in any one of claims 1-6 and 16.

18. A process for combatting insects which comprises exposing them to proteins or compositions as claimed in any one of claims 1-6, 16 and 17 or the micro-organism of claim 14.

19. An extraction process for obtaining insecticidal proteins, as claimed in any one of claims 1-6 or claim 16, from organic material containing them comprising submitting the material to maceration and solvent extraction, characterized in that the material is a microorganism.

cry1Ba

1445

CTTGGACGCATCGTAGTGCCAGATCGTACCGAATACGATTGGACC

* *

5' GGACGCATCGTAGTGCCGGACCGTACCGAATACGATTGG 3'*cry1Ia*

1469

CTTGGACGCATCGTAGTGCCAGATCGTACCAATACCAATTGAG

* *

5' GGACGCATCGTAGTGCCGGACCGTACCAATACCAATTG 3'

FIG. 1

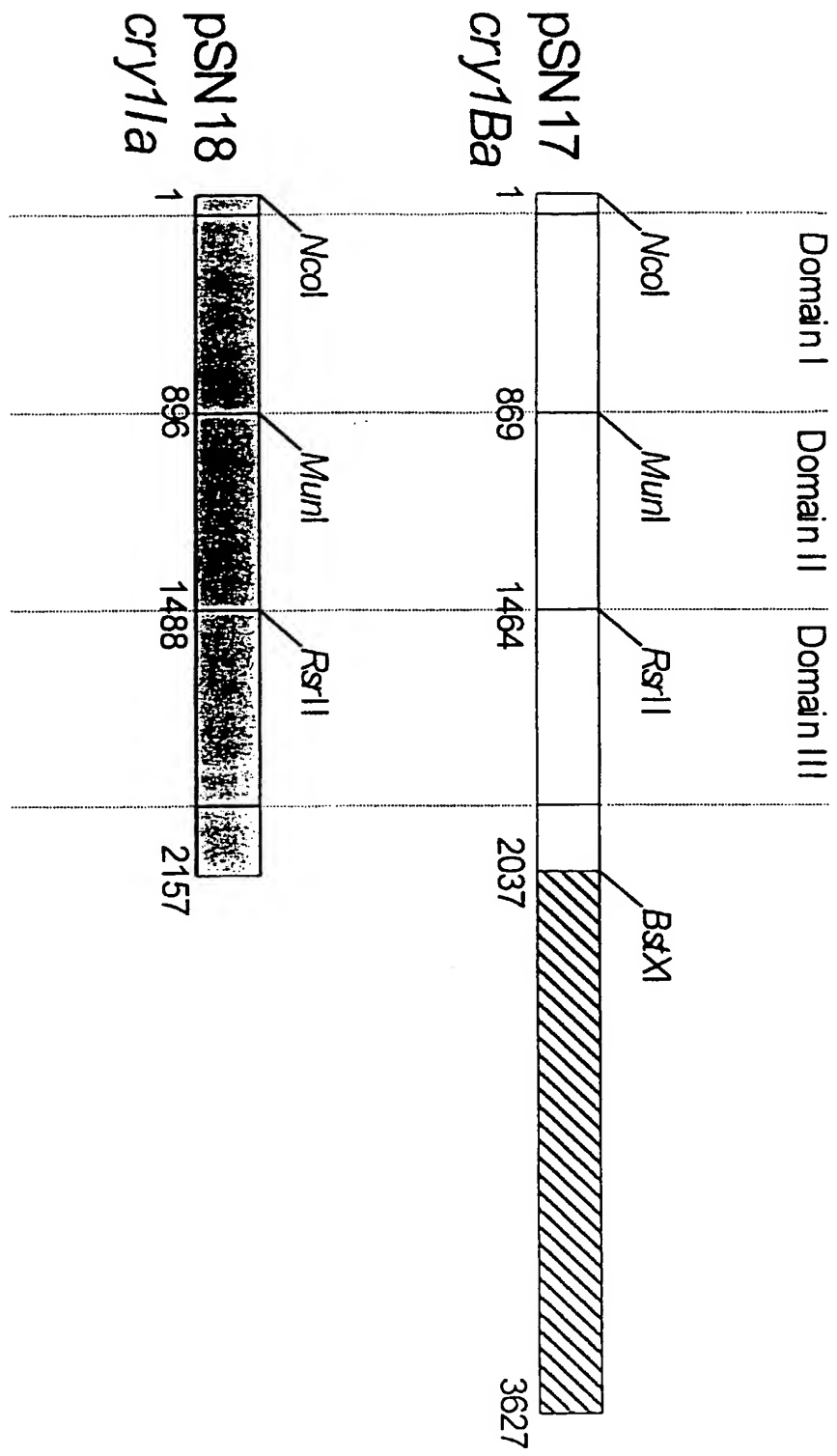


FIG. 2

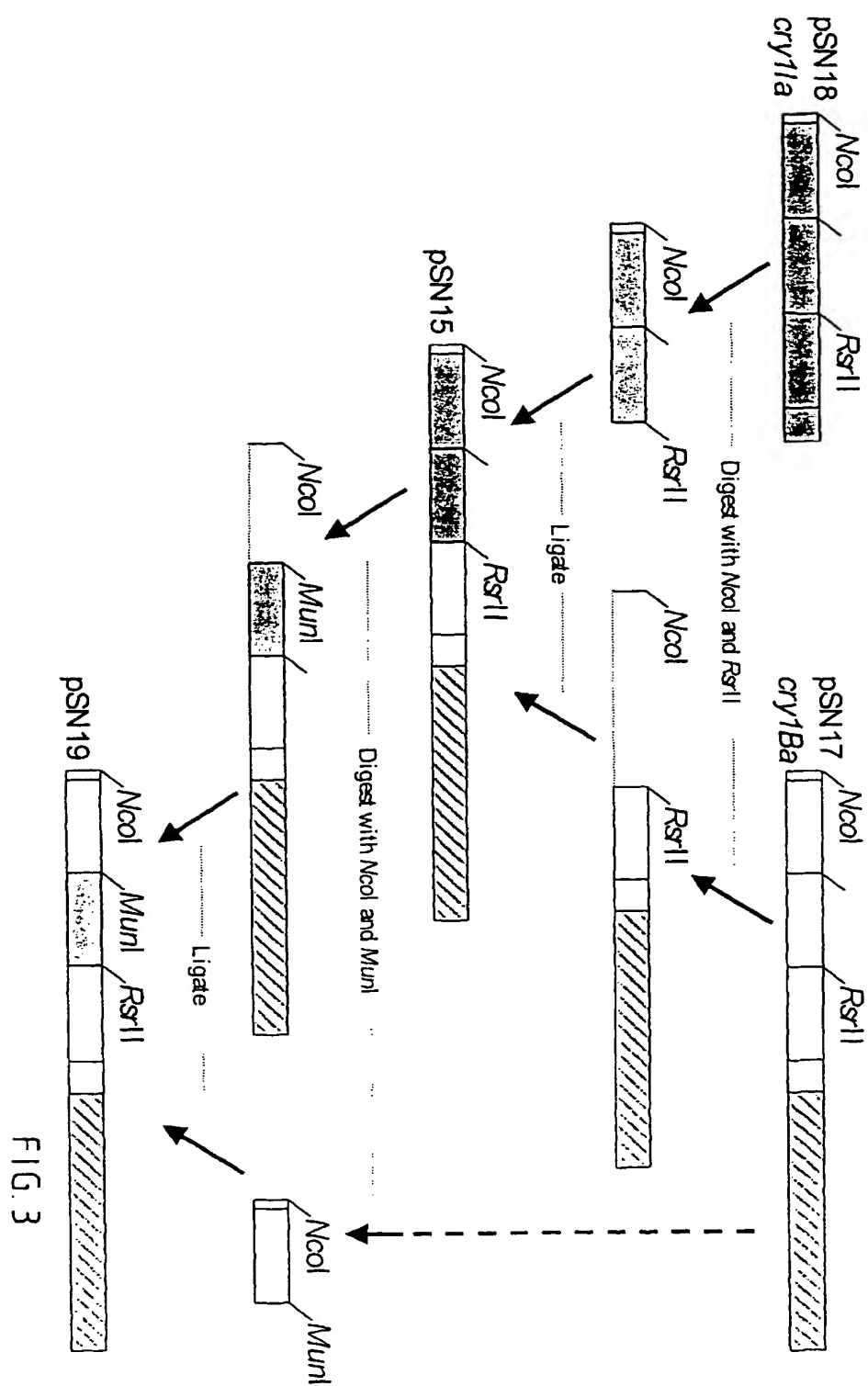


FIG. 3

A

RsrII

cry1Ia 1471 TGGACGCATCGTAGTGGC.....a.....a....
cry1Ba 1447GACCGTACCGAATACGATT
 TrpThrHisArgSerAlaAspArgThrAsnThrIle

B

MunI

cry1Ba 853 GAAGTTTATACAGACGC.....ag..ac.ggggtta
cry1Ia 880a.....AATTGGGACAGTACATCCG
 GluValTyrThrAspAlaIleGlyThrValHisPro

FIG. 4

Application Number
EP 99 20 3723



European Patent
Office

Application Number
EP 99 20 3723

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☒ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 20 3723

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (InCL1.7)
D,A	BRADLEY D ET AL: "The Insecticidal CryIB Crystal Protein of Bacillus thuringiensis ssp. thuringiensis Has Dual Specificity to Coleopteran and Lepidopteran Larvae." JOURNAL OF INVERTEBRATE PATHOLOGY, vol. 65, no. 2, 1995, pages 162-173, XP000891272 ISSN: 0022-2011 ---		
D,A	MAAGD DE R A: "DIFFERENT DOMAINS OF BACILLUS THURINGIENSIS DELTA-ENDOTOXINS CAN BIND TO INSECT MIDGUT MEMBRANE PROTEINS ON LIGAND BLOTS" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 62, no. 8, August 1996 (1996-08), pages 2753-2757, XP002061801 ISSN: 0099-2240 -----		
			TECHNICAL FIELDS SEARCHED (InCL1.7)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 20 March 2000	Examiner Andres, S
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03.02 (P/ALC01)



European Patent
Office

**LACK OF UNITY OF INVENTION
SHEET B**

Application Number
EP 99 20 3723

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-18

Hybrid *Bacillus thuringiensis* insecticidal toxins comprising a domain II derived from CryIIa and a domain III derived from CryI8a. Nucleic acids encoding the toxins, vectors containing them, plants transformed therewith, and compositions containing the proteins.

2. Claim : 19

An extraction process for obtaining insecticidal proteins.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 99 20 3723

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

20-03-2000

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9950293	A	07-10-1999	AU	3148099 A	18-10-1999

WO 9506730	A	09-03-1995	AU	691522 B	21-05-1998
			AU	7693294 A	22-03-1995
			BR	9407377 A	16-07-1996
			CA	2168011 A	09-03-1995
			EP	0719335 A	03-07-1996
			JP	9502343 T	11-03-1997
			PL	313317 A	24-06-1996
			US	5736131 A	07-04-1998
			ZA	9406770 A	04-03-1996

EPO FORM P0159

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

